# Programmed for sex: Nutrition–reproduction relationships from an inter-generational perspective

REPRODUCTION

SEX IN THREE CITIES REVIEW

# Programmed for sex: Nutrition–reproduction relationships from an inter-generational perspective

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## Abstract

**Reproduction is our biological reason for being. Our physiology has been shaped via countless millennia of evolution with this one purpose in mind, so that at birth we are 'programmed for sex', although this will not kick-start functionally until puberty. Our development from an early embryo is focused on making us fit to reproduce and is intimately connected to nutrition and energy stores. Fluctuations in food supply has probably been a key evolutionary shaper of the reproductive process, and this review hypothesizes that we have developed rapid, non-genomic adaptive mechanisms to such fluctuations to better fit offspring to their perceived (nutritional) environment, thus giving them a reproductive advantage. There is abundant evidence for this notion from 'fetal programming' studies and from experimental 'inter-generational' studies involving manipulation of parental (especially paternal) diet and then examining metabolic changes in resulting offspring. It is argued that the epigenetic reprogramming of germ cells that occurs during fetal life, after fertilisation and during gametogenesis provides opportunities for sensing of the (nutritional) environment so as to affect adaptive epigenetic changes to alter offspring metabolic function. In this regard, there may be adverse effects of a modern Western diet, perhaps because it is deficient in plant-derived factors that are proven to be capable of altering the epigenome, folate being a prime example; we have evolved in tune with such factors. Therefore, parental and even grandparental diets may have consequences for health of future generations, but how important this might be and the precise epigenetic mechanisms involved are unknown.**

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## Introduction

We live in an age when reproduction is not the force in our lives that it was historically. Not much more than a century ago, average family sizes/birth rates were huge by modern day standards, even if the reasons for this (need children for family support, high child mortality, short life expectancy, poor contraception) may appear rather alien to us in the modern, developed world. Consequently, back in these times, fertility was of central importance in most people's lives, in ways that are far less obvious today. In the Western world, and increasingly in the developing world, our perception now is that reproduction (i.e. fertility) is at our beck and call – we can turn it off and on (in women) when it suits us, a view reinforced by the development of a battery of assisted reproduction techniques for when things are not so straightforward on the fertility front. Therefore, it is easy to see how we have come to consider ourselves as being in control of our reproduction rather than reproduction being in control of us. Such thought processes have unfortunately permeated into biomedical research and have played a role in downgrading the importance of 'reproductive research' and its priority for funding. In this review, I hope to show that our present perspectives are divorced from reality and that, whether we appreciate it or not, reproduction is very much in control of us, and this control permeates almost every aspect of our lives. Conversely, any major changes to our lifestyle and environment, in particular to our diets and metabolism, have the potential to impact reproduction, and perhaps also the future health and wellbeing of our children and grand-children.

Over millennia, evolution has shaped us as we are today with one purpose in mind – to reproduce. It is our biological reason for being. Our DNA is immortal and will persist (through our children), whereas our bodies (i.e. ourselves) are simply short-lived carriers of that DNA – what could be viewed as evolutionary 'pass the DNA parcel' (but with ultimate sequential fatality for the players). As a consequence, all of our development and function are geared towards the purpose of reproduction. Once you accept this premise (and unfortunately not many do) you learn to view our physiology and development through the prism of reproduction. This formed the basis for my 'Sex in three cities' series of talks and for the discussion and hypotheses offered below.


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The aim is to get the reader to think about reproduction as a long-term investment and to consider whether nature has evolved epigenetic regulatory mechanisms to enable rapid adaptation of future offspring to their perceived nutritional environment in order to gain a long-term reproductive advantage.

## Sex and reproduction are at the forefront during development

It is understandable for us to think that, until we hit puberty, sex and reproduction do not matter and play no role in our lives, because from a purely functional perspective this is correct. However, once successful fertilization has occurred and 'reproduction is underway', nature's focus is on sex and reproduction for the new conceptus almost straight away. Thus, soon after implantation of the new embryo and before any recognizable body-plan is in place, the primordial germ cells (=future reproduction) are set aside like 'crown jewels' (Johnson & Alberio 2015, Canovas *et al.* 2017) in what can be viewed as the first definitive cell differentiation of the body to occur during development. Moreover, as soon as a body-plan is in place, for what can now be termed a fetus, one of the very first events is to decide on which sex the fetus is to become and to orchestrate the development of a complete reproductive tract and gonad appropriate for the sex of the fetus. In humans, this is more or less completed by around 12–14 weeks of gestation when the fetus is only 3–5 cm in length. It is becoming increasingly evident, in males at least, that much of the reproductive dysfunction that becomes apparent in young adulthood may originate because of faulty programming (by androgens) during this early fetal period – the so-called testicular dysgenesis syndrome (TDS). For space reasons, this will not be discussed here, but interested readers can refer to relevant publications for details (Dean & Sharpe 2013, Skakkebaek *et al.* 2016, van den Driesche *et al.* 2017).

In contrast to gonad and reproductive tract differentiation, sexual differentiation of the brain, including the programming of sex-specific reproductive behaviours is programmed later in gestation, probably in the second and third trimester in humans (Swaab 2007). Thus, a functional reproductive system is fully in place by birth in both sexes in humans, although it normally remains dormant until it is activated (by hormones) during puberty; however, such activation can be triggered prematurely at any time after birth, which is termed precocious puberty (Leka-Emiri *et al.* 2017). In the context of the present article, the point to be made is that a 'reproductive plan' is in place from the earliest points of embryonic and fetal development, hence, the notion that we are 'programmed for sex (i.e. for reproduction)' from the moment we are born. As will be argued throughout the rest of this article, a more sophisticated aspect of this plan might involve adjusting aspects of reproductive and/or physiological (e.g. metabolic) development to the perceived environment (primarily nutrition/food supply) into which it will be born, with the aim of giving the individual a reproductive advantage (Fig. 1). This is not a wild idea, because it is already well established that most key aspects of the reproductive process in both sexes are closely attuned to the environment and to energy supply.

## Interplay between energy stores, metabolism and reproduction

Long-term reproductive success requires that reproductive processes are attuned to the environment, in particular to the food/nutrient supply. It is for this reason that most mammals are seasonal breeders, they reproduce (gametogenesis, sexual behavior/mating) such that it will lead to birth of the resulting offspring at a time of year when food will be abundant (usually the spring). In such animals, photoperiod is the most important cue used to time the seasonal 'switching on and off' of reproductive processes in both sexes (Malpaux *et al.* 2001, Henningsen *et al.* 2016). Such cues are used not only to control reproduction but also to control appetite to ensure that, for example, species in which a pregnancy is carried over the winter have accumulated enough fat (energy) stores to support fetal growth (Clarke *et al.* 2000); similar seasonal changes also occur in males (Lincoln *et al.* 2001). As well as optimizing reproduction, this close attunement to the environment provides mechanisms via which the species can potentially adapt reproduction to alterations in the food supply. Although humans are not seasonal breeders, we evolved from such a background and there are clear echoes of seasonality in, for example, birth and twinning rates (Rojansky *et al.* 1992, Cummings 2014, Sharpe 2017) and in sperm counts in men (Jorgensen *et al.* 2001, Sharpe 2017). Moreover, in general, it is the same processes that regulate normal puberty as regulate seasonal activation of reproductive function in seasonal breeding species; the latter can therefore be considered as 'seasonal puberty' (Smith & Clarke 2010).

There is now general recognition that timing of reproductive function onset (puberty) is orchestrated by a push–pull system in the hypothalamus involving factors that lead either to suppression or stimulation of GnRH secretion, which in turn regulates gonadotrophin synthesis and secretion and their effects on growth and function of the gonads (Wahab *et al.* 2015). The same systems are also used to control seasonal reproduction in seasonally breeding mammals, although there can be species-specific refinements to the precise control mechanisms (Smith & Clarke 2010, Henningsen *et al.* 2016). Current thinking (Fig. 2) is that GnIH (gonadotrophin-inhibiting hormone; also termed RFRP-3)

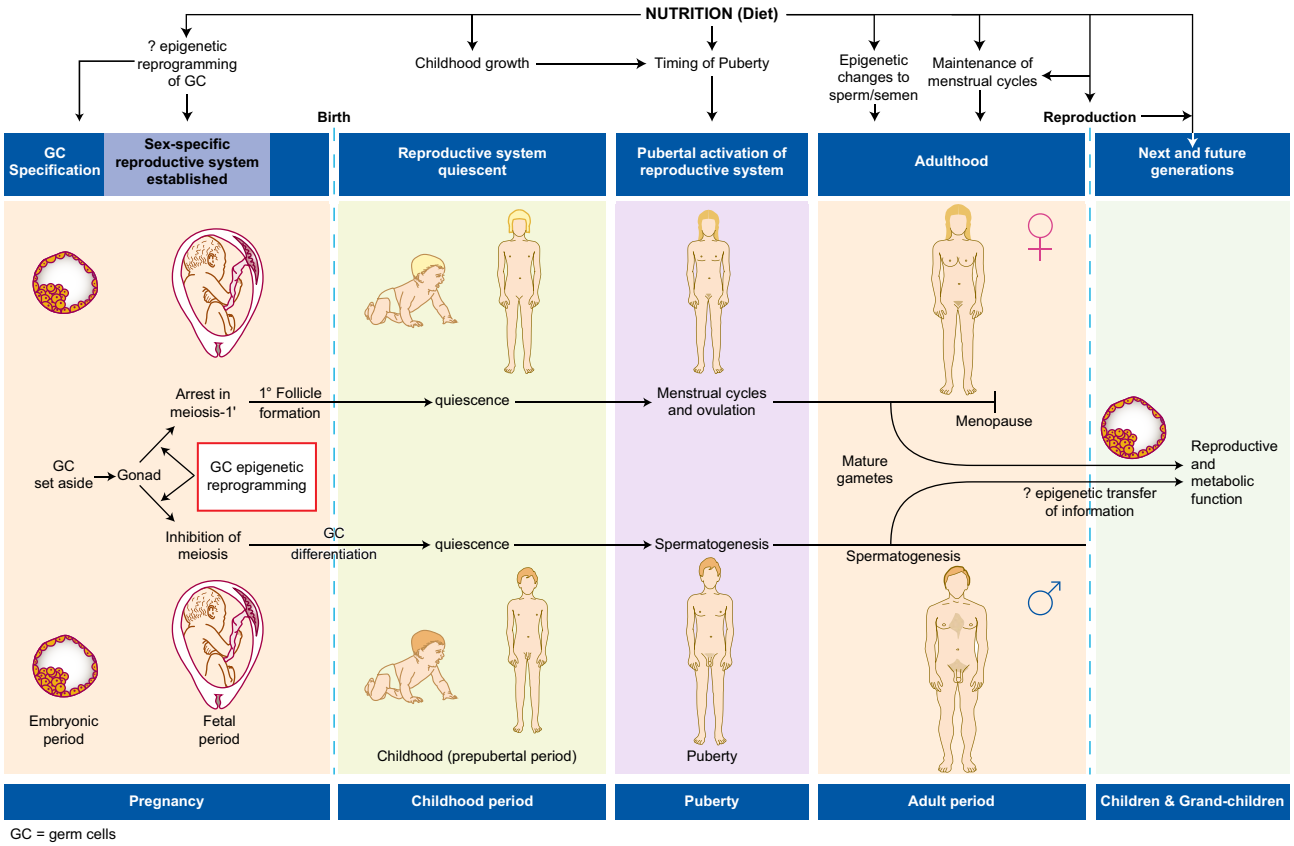


**Figure 1** Key steps in reproductive development and function during the life-course and the points at which nutrition can impact this process. The potential epigenetic impacts are discussed in detail in the text.

exerts negative control of GnRH secretion, whereas kisspeptin (KP) exerts positive control (Smith 2012, Wahab *et al.* 2015), although time may reveal that there are also other modulatory pathways. In parallel to effects on GnRH, GnIH and KP also exert important effects on appetite control, based on a variety of experimental studies (Wahab *et al.* 2015). Thus, GnIH is oroxigenic (appetite-stimulating), ensuring that further energy stores will be built up whilst reproduction/puberty onset is suppressed, whereas KP is anorexigenic (appetite-suppressing). Although it may seem odd that KP is anorexigenic, it needs to be kept in mind that activation of the KP system is dependent on signals within the body that sufficient energy (fat) stores have been accumulated to support puberty and, in females, pregnancy; thus, further stimulus to *increase* appetite is no longer needed. Recent evidence also points to a role for KP in promoting sexual and couple-bonding behavior in human males (Comninos *et al.* 2017), demonstrating how all aspects of the reproductive process are linked mechanistically to nutritional/energy store-modulated factors.

As with animals, it is likely that the driving force behind seasonal influences on human reproduction, from an evolutionary perspective, is fluctuations in food availability, although it has to be recognized that this can exert effects by impacting infant mortality as well as birth rate (Hayward & Lummaa 2013). However, if reproduction is 'our reason for being', then our reproductive lives should also show other manifestations of the influence of food/nutrition and evidence for our attunement. This can be illustrated by examining the effect of nutrition on timing of the age of menarche (=puberty) in girls, because this is arguably the pivotal event in human reproduction (Fig. 1). To ensure reproductive success, girls need to have acquired certain essential physical features prior to initiation and completion of puberty, namely sufficient stature and skeletal development to support a pregnancy (Villamor & Jansen 2016). Even when this box is ticked, it is probably nutritional status that finally calls the shots, as it is essential that the female has sufficient energy (fat) stores to support both the pregnancy and the following lactation. If energy stores fall below a certain threshold, such as in women with anorexia, then the reproductive axis is essentially switched off (Kaplowitz 2008). In the human context, sufficient social and emotional development are probably also important for reproductive success, but this is an aspect of considerable complexity (see Gluckman & Hanson 2006) and will not be considered further here.

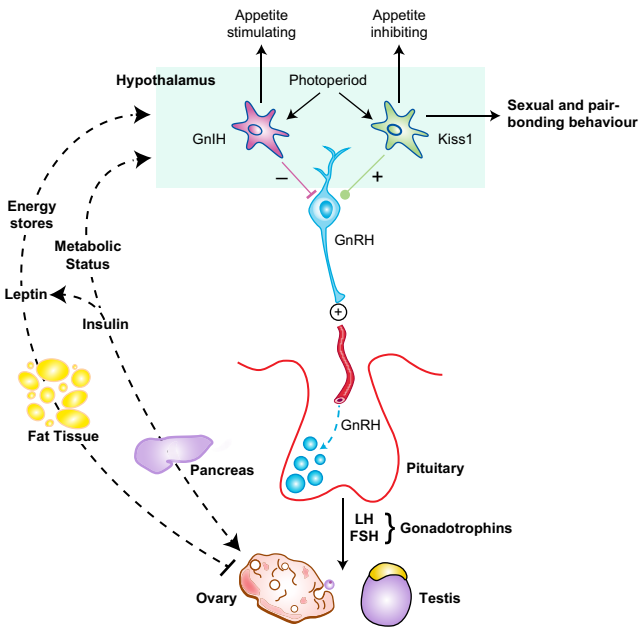


**Figure 2** Principal components of the interface between the metabolic system and adipose tissue/energy stores and activation of the reproductive system at puberty. GnIH, gonadotrophin-inhibiting hormone; GnRH, gonadotrophin-releasing hormone; Kiss1, kisspeptin.

Acquisition of sufficient energy stores is important in initiating female puberty for the reasons just outlined. By depositing these fat stores in breast tissue and around the thighs and buttocks in young women, this also acts as a signal to males that the woman has attained reproductive age, and is thus 'fit for reproduction'. The clearest demonstration of this is that when there is no intact leptin signal from fat stores, due to mutations in the leptin gene or its receptor, then puberty fails to occur (Kaplowitz 2008). Moreover, in normal girls, leptin levels are positively associated with age at menarche (Matkovic *et al*. 1997) and high leptin levels are associated with earlier menarche independent of fat stores (Gavela-Perez *et al*. 2016). Indeed, improved nutrition has undoubtedly been the key factor in determining the decrease in age at menarche in girls that has occurred since the mid-1800s, although this has stabilized in developed countries since the mid-1900s (Sorensen *et al*. 2012, Biro & Kiess 2016).

In contrast to females, the evidence for nutrition and fat stores playing a role in male puberty is less evident, probably because acquisition of adult reproductive function in males is not especially energy demanding, as it is in females. Nevertheless, carefully designed studies have provided some evidence for recent advance in the age of puberty in boys, associated with nutritional status/BMI, although the changes involved appear more modest than those that have occurred in girls (Sorensen *et al*. 2010, Biro & Kiess 2016). An interesting issue that such sex differences raise is whether, historically, age of puberty was comparable in girls and boys and has become increasingly disparate since the 19th century. However, as puberty timing in boys is far harder to define than in girls, accurate historical data is not easily available.

From the brief outline above, it is clear that nutrition plays a key role in the reproductive process whether in seasonal or non-seasonal breeders (e.g. humans). It seems obvious why this should be the case in females, especially for puberty timing, because pregnancy and the following lactation are critically dependent on adequate nutrition and energy supplies, and a firm foundation for successful reproduction is essential – and thus the fulfillment of nature's plan. However, it is equally evident that the same seasonal/environmental cues that drive appetite and energy intake in females also do the same in males (Lincoln *et al*. 2001, Anukulkitch *et al*. 2007), perhaps because breeding and associated spermatogenesis may also be energy-demanding, albeit on a different scale to the female. The important take-home message is that both sexes are in tune with their environment and responsive to changes in appetite, which is in turn attuned to the food supply (Fig. 1). This being the case, it is not a huge leap of the imagination to consider that such attunement could influence more than the timing of puberty/seasonal breeding. Given the importance of successful reproduction in the long-term survival of any species, and the fact that reproduction is by definition 'inter-generational', might attunement of reproduction to food supply/nutrition also operate in an inter-generational context? I attempt to make a case for this in the rest of this article.

### *Reproduction and natural selection*

We are all familiar with this concept, which originated with Darwin as 'survival of the fittest' and was then modified into the concept of natural selection. Sexual reproduction plays the deciding role in this process in two fundamental ways. First, natural selection presumes that only the fittest will survive to reproduce or will reproduce the most, hence providing the basic pivot for the whole process. Second, because of the recombination events during the processes of gametogenesis and then fertilization, reproduction provides the literal breeding ground for new genomes that may prove to be better adapted to any change in the environment or to a new environment or niche. One can see how this could work over huge spans of time, but it seems far too risky if the requirement is to enable adaptability of a species to a rapid environmental change such as food availability. Indeed, it amounts to little more than a random throw of the genomic dice which, circumstantially, might turn out to be an advantage or a disadvantage to survival of that individual if its environment changed.

When viewed through the reproductive prism, it seems odd that the huge investment that has been made over millennia in attuning of reproduction to the environment in a given species, could be based on literal

'reproductive roulette' via a throw of the genomic dice. Frankly, this seems unfit for purpose, which convinces me that nature would have evolved far better ways of adapting (quickly) without having to change the DNA itself – by using epigenetic mechanisms. Against this view is the undisputed fact that numerous species have become extinct over time, usually in the face of dramatic environmental change, so why did they not adapt epigenetically? Of course, there are limits to adaptability of any species – dinosaurs could not have adapted to the calamitous change in their environment that wiped them out. But adaptation to much more common, but less dramatic changes, for example, in seasonal food supply, would make survival and evolutionary sense. Moreover, such adaptive changes would be most effective if they were fast (i.e. not requiring multiple generations to be effective) and flexible (e.g. easily reversible), features that are simply not compatible with natural selection based on changes in DNA sequence. In contrast, epigenetic changes within germ cells can theoretically offer such possibilities, and the evidence that such mechanisms might be operative is discussed below.

### *Epigenetic adaptation and reproduction*

From a purely theoretical point of view, there are several distinct periods during the reproductive process when epigenetic adaptive changes could be induced to better fit resulting offspring to a changed environment (Box 1); here, we will simply focus on evidence related to changes in nutrition/food supply, but in theory, adaptive changes to other environmental factors such as stress (Bale 2014) could also be important. First, the developing or maturing gametes could be affected by nutrition of the individual. Second, the zygote/early embryo could be affected, primarily by nutrition of the mother but perhaps also by the father's nutrition. Third, the developing fetus could be affected, primarily by maternal nutrition. Fourth, the resulting offspring could be affected by nutrition at one or more points during the life course – this will not be considered here. There is a substantial body of experimental evidence for effects during the first three of these periods, which are summarised below. However, before dealing with this evidence, it is appropriate to first comment on several major issues to be kept in mind when considering and interpreting this evidence. These relate to the nature of the evidence, whether 'effects' have been induced via maternal or paternal 'exposures', and the mechanism for such effects; in many ways, these three aspects are inter-related.

The first point is that the majority of the evidence for adaptive changes in offspring with a presumptive epigenetic basis is indirect – it is based on changes in obesity and/or metabolic pathways or disease susceptibility in the offspring without evidence that this is the direct effect of a nutritionally induced epigenetic change at some point during the origin/development of these individuals. Therefore, an epigenetic adaptive change has been presumed rather than demonstrated, and in some cases, alternative explanations may not have been considered or excluded. The second point is whether the presumptive adaptive change has been induced as a consequence of an experimental change in nutrition of the mother or father. If the mother's nutrition was changed does this lead to changes in the offspring via an altered uterine environment that, for example impacts placental function, or because of an epigenetic change in the offspring? This issue is especially important when considering the inter-generational transmission of 'effects', as discussed further below. It is more likely that a change in the offspring has an epigenetic basis if it results from a specific dietary change in the father, because there is no potential confounding of the uterine environment involved. However, it is still possible with paternally mediated effects that alternative pathways could be involved, for example, via transmission of factors (e.g. miRNAs) via seminal plasma that then impact early embryo development without any direct transfer of epigenetic changes via the sperm chromatin itself (Daxinger & Whitelaw 2012, Binder *et al.* 2015). Of course, this example still ultimately involves an epigenetic mechanism, but not one involving the sperm epigenome itself. Finally, the third point is that in nearly every experiment in which a presumptive epigenetically mediated transmission of a phenotypic change to offspring has been transmitted, the actual epigenetic mechanism has not been elucidated.

The brief outline of the above 3 points reminds us that, unlike with DNA-mediated inheritance, epigenetically mediated inheritance is far more complex to unravel, pin down and study. Although this is due in part to our present ignorance about epigenetic regulatory mechanisms, it is also an inherent feature of epigenetics. An epigenetic change can be ephemeral, it may cause its effect in the offspring and then disappear, leaving only the downstream consequences of the initial effect visible; at least with DNA mutations/polymorphisms, the causal evidence always remains. As a consequence, most of the studies involving putative inter-generational epigenetic effects after experimental intervention, are based on demonstrating phenotypic changes in the offspring, compared with relevant controls.

**Box 1 Opportunities for induction of specific epigenomic changes in response to the nutritional environment (or diet).**

- During gametogenesis – especially during spermiogenesis in males
- During epigenetic reprogramming of primordial/fetal germ cells
- During genome-wide reprogramming post fertilisation (in pre-implantation embryos)
- During fetal development (e.g. organogenesis)

### *Offspring effects resulting from dietary changes during parental gametogenesis*

There are numerous studies which have shown that experimental manipulation of the diet in rodents during puberty/adulthood can impact the next generation (Wei *et al.* 2015). Most studies have focused on the impact of parental obesity and/or a high-fat diet (HFD), and in general, these show that this results in increased risk of obesity, metabolic dysfunction and pre-diabetic changes in the offspring, although in some studies only male offspring are affected. The majority of studies have focused on paternal obesity/HFD (Box 2) because this avoids any confounding 'uterine effects' that are possible with maternal exposures and have shown adverse metabolic and reproductive changes in offspring of such fathers (Ng *et al.* 2010, Palmer *et al.* 2011, Fullston *et al.* 2013, McPherson *et al.* 2014); some studies have shown that such 'programmed' effects are transmissible to further generations (Fullston *et al.* 2012, King *et al.* 2013). These paternally transmitted effects are not just triggered by 'over-nutrition' because feeding males on a low protein diet prior to mating, also results in altered hepatic expression of a range of genes involved in lipid and cholesterol metabolism in offspring sired by such males (Carone *et al.* 2010). One of the more interesting findings is that the next-generation effects of paternal obesity in mice can be reversed by altering the diet of the fathers or by getting them to increase their exercise levels (Palmer *et al.* 2011, McPherson *et al.* 2014, Barres & Zierath 2016). A study of obese men before and after bariatric surgery provides support for this (Donkin *et al.* 2015). These authors showed, first, that sperm from lean and obese men showed differences in DNA methylation and in small non-coding RNAs, and second, that obese men who underwent bariatric surgery showed dramatic remodelling of sperm DNA methylation, notably at genetic locations implicated in central control of appetite.

**Box 2 Evidence that a modern Western diet in fathers can 'reprogram' future offspring.**

- Experimental rodent dietary intervention studies
  - cause epigenetic changes to sperm
  - result in metabolic changes in offspring
  - these effects are dynamic and reversible
- During histone replacement in spermiogenesis, specific regions containing key development/metabolic genes retain nucleosomes
- Numerous plant-derived products in a balanced diet have the potential to modulate the epigenome
- A Western style diet may be deficient or unbalanced in these 'epigenetic modulators' (e.g. folate)

A key, presently unanswered question, is whether the experimental dietary studies in adult male mice can be translated to human males. Human cohort studies have been used to try and assess relationships between parental obesity/BMI and offspring BMI, but the results obtained have been quite mixed. Two studies have reported a significant association between paternal and offspring BMI (Birbilis *et al.* 2013, Linabery *et al.* 2013), and both studies also showed a significant association between maternal BMI and offspring BMI, although the studies differed as to which parent's BMI had the strongest association with offspring BMI. A systematic review, which only identified three suitable cohort studies with full data, found that no strong conclusions could be reached based on present data (Patro *et al.* 2013). Whether this means that inter-generational effects of obesity are less evident in humans than in experimental rodent studies or whether the large number of potential confounding factors in human studies, especially with maternally mediated 'effects', creates too much noise for inter-generational effects to be discernible, is a matter for debate and further investigation. In this regard, one interesting study, which is discussed in detail later in this review, has shown that variation in methylation of the pro-opiomelanocortin (*POMC*) gene in neurons involved in appetite control is strongly correlated with BMI in individual humans (Kuhnen *et al.* 2016). This is significant because methylation of this gene is determined embryonically and can be affected by peri-conceptual diet of the mother as well as by epigenetic transmission from the father (Kuhnen *et al.* 2016).

The aforementioned mouse and human studies indicate that whatever the epigenetic mechanism underlying the inter-generational effects of paternal obesity, they are dynamic and can adapt to alterations in the father's nutritional environment (Box 2). However, the big unanswered question is what is the epigenetic mechanism for these dynamic inter-generational effects? The honest answer is that, at present, there is a lack of examples to show that a diet-induced specific epigenetic change(s) in germ cells in one generation is demonstrably transmitted to, and responsible for, an observed consequence in the next generation(s); logistically, such studies are extremely challenging. Nevertheless, there are a growing number of studies that show how paternal diet can cause epigenetic changes in sperm, for example, via altering the miRNA profile (Fullston *et al.* 2013, Grandjean *et al.* 2015, Sharma *et al.* 2016), chromatin structure/histone modifications (Carone *et al.* 2010, Ost *et al.* 2014) or the DNA methylome (Radford *et al.* 2014) of spermatozoa (Box 2). However, when in spermatogenesis such effects are induced, and whether these germline epigenetic modifications are directly responsible for phenotypic changes in offspring are unknown.

### *Offspring effects resulting from maternal dietary changes during embryogenesis*

There is abundant experimental evidence from mouse and sheep studies that show that altered maternal nutrition during the peri-conceptual pre-implantation period can result in adverse metabolic function/pre-diabetic changes as well as size, obesity, altered blood pressure, behaviour and adrenal function in the resulting offspring in later life (Grace & Sinclair 2009, Fleming *et al.* 2012, 2015; Box 1). Most of these studies have focused on maternal protein under-nutrition, but it also appears that over-nutrition/obesity may cause similar effects (Fleming *et al.* 2012). As indicated earlier, studies in pregnancy are subject to potential confounding from uterine environment effects, but the mouse studies have sidestepped this problem by flushing the embryos from mothers that had been exposed to a low protein diet just during the peri-conceptual period, and then implanting them into the uterus of mothers fed a normal control diet. When these resulting offspring grew up they exhibited comparable dysfunction to offspring that had continued development in the uterus of mothers who had been exposed peri-conceptually to a low protein diet (Watkins *et al.* 2008). It appears that one early response to exposure of the mouse embryo to a low protein diet is to cause increased proliferation of the trophectoderm and its outgrowths during implantation (Eckert *et al.* 2012) as well as functional changes to nutrient transport mechanisms (Watkins *et al.* 2008, Fleming *et al.* 2015). Another study has shown that a maternal low protein diet just around the time of conception results, in offspring, in excessive ribosomal DNA (rDNA) transcription and ribosome biogenesis when nutrition after birth is plentiful (Denisenko *et al.* 2016), providing a plausible mechanism to explain increased risk of obesity ('overgrowth') in such individuals. Indeed, the authors suggest that *rDNA* is a plausible candidate for a 'thrifty gene involved in nutrient ulitization control, which is tuned by nutrient availability *in utero*'. Adaptive changes such as these are considered to sow the seeds for metabolic and other dysfunctions in adulthood (Fleming *et al.* 2015, Xu & Sinclair 2015).

The precise epigenetic mechanisms that underlie the various phenotypic changes in offspring affected as a result of maternal peri-conceptual diet remain to be determined, but studies of mouse embryoid bodies derived from cell lines from blastocysts from mothers exposed to normal or low protein diets peri-conceptually, show diet-dependent histone changes that are propagated during cell divisions, which may be involved (Sun *et al.* 2015). Studies focussed on the *rDNA* gene and how its expression may be altered by peri-conceptual diet of the mother, could be particularly informative as results already point to altered gene methylation as being important (Denisenko *et al.* 2016). In this regard, it is already apparent that 1-carbon metabolism, which plays an essential role in DNA methylation may be an important pathway of effect for peri-conceptual changes in diet etc. (Xu & Sinclair 2015), as is discussed in more detail below.

One particularly important implication of the experimental mouse and sheep embryo studies is in regard to assisted reproduction techniques (ART) in humans, as these routinely require short-term culture of early conceptuses. If the animal studies are any guide, differences in the 'nutrients' in the culture medium employed (e.g. folic acid) could potentially induce epigenetic changes that alter later development and function of the embryo and/or placenta, and whatever downstream health consequences these might trigger (Grace & Sinclair 2009, Steegers-Theunissen *et al.* 2013). There is growing evidence for such effects in humans (Steegers-Theunissen *et al.* 2013, Sunde *et al.* 2016), and this area is likely to attract increasing attention as our understanding of the vulnerable or adaptive epigenetic mechanisms in the early embryo are identified.

### *Offspring effects resulting from maternal dietary changes during the fetal period*

The term 'fetal programming' has been used extensively as a catch-all for describing the adverse health consequences that result from fetal growth restriction, whether this be due to impaired placental development and/or function, to maternal under-nutrition or to maternal over-nutrition or HFD, which can adversely impact placental function (Huypens *et al.* 2016, Musial *et al.* 2017). This leads to increased risk of obesity and metabolic syndrome disorders when the growth-restricted fetus becomes an adult; such effects have been extensively described in humans, domestic and laboratory animals (Desai *et al.* 2015, Reynolds *et al.* 2015, Aiken *et al.* 2016*a*, Cheong *et al.* 2016) and will only be outlined in general here. One interpretation of such findings is that, irrespective of the reason for fetal growth restriction, it is perceived by the fetus as evidence of a poor nutritional environment in the world into which it will be born, which triggers metabolic changes to better adapt it to this environment – the so-called 'thrifty phenotype' hypothesis (Hales & Barker 2012). However, when such growth-restricted fetuses are born into a world of normal nutrition or, increasingly, one of over-nutrition (Western diet), their inappropriate metabolic reprogramming leads to over-compensation of growth (especially in the immediate postnatal period) and consequential increased risk of obesity, metabolic dysfunction/type II diabetes and all of the downstream adverse health changes that these changes bring, including cardiovascular disease (Alexander *et al.* 2015). These (mal)adaptive metabolic changes must have an epigenetic basis, and various such changes have been described in different such models (Vickers *et al.* 2011,

Desai *et al.* 2015, Banik *et al.* 2017). Of interest is that the maladaptive changes can include reprogramming of central appetite regulation (Martin-Gronet *et al.* 2016), pancreatic β-cell mass (Portha *et al.* 2011), liver metabolic function and adipose tissue effects (McMillen *et al.* 2008, Vickers *et al.* 2011), demonstrating that the effects are very much a 'cover-all' for modulation of food intake, energy metabolism and storage, rather than being targeted to a single aspect.

From the reproductive perspective, the adverse health effects of nutrition-induced fetal reprogramming means that the individual is less fit to reproduce in general terms. However, there may also be more specific effects. For example, it has been shown in rodent models that feeding the mother a low protein diet or a Western style obesogenic (high fat/high sugar) diet during pregnancy, both result in reduced ovarian reserves in female offspring (Aiken *et al.* 2015, 2016*b*). Interestingly, other rodent studies have shown that similar effects are induced by maternal exposure to analgesics during pregnancy (Dean *et al.* 2016, Holm *et al.* 2016), probably involving a prostaglandin E2-mediated pathway (Dean *et al.* 2016). If similar effects of maternal diet or analgesic exposure occur in human pregnancy, it could have significant impact on female reproductive lifespan, but designing relevant studies in humans is extremely difficult because of their complexity and duration (Aiken *et al.* 2016*a*).

### *Intergenerational effects: relationship to fetal germ cell reprogramming*

In rodents, fetal primordial germ cells go through a process of widespread genomic demethylation during and following their migration into the genital ridge/gonad, followed some days later by remethylation in a sex-dependent manner (earlier in males than in females, where it occurs postnatally) (Sasaki & Matsui 2008, Seisenberger *et al.* 2012, Kobayashi *et al.* 2013) (Fig. 1, Box 1). At the same time, dynamic changes in histone methylation and in other histone modifications also occur (Seki *et al.* 2007, Sasaki & Matsui 2008, Prokopuk *et al.* 2017). This reprogramming process is thought to be essential for later sex-specific germ cell development, including pluripotency changes and entry to meiosis (Reik 2007, Sasaki & Matsui 2008, Seisenberger *et al.* 2012, Guo *et al.* 2017). The more limited evidence available for the human fetus demonstrates that similar DNA methylation and histone changes also occur (Biermann & Steger 2007, Wermann *et al.* 2010, Tang *et al.* 2015*a*,*b*, Guo *et al.* 2017), these changes taking place during weeks 5–7 of gestation (Tang *et al.* 2015*a*,*b*); however, there may be some differences between mouse and human as to how these reprogramming changes are affected (Tang *et al.* 2015*a*,*b*, 2016, Guo *et al.* 2017). Viewed through the reproductive prism, the fetal germ cell epigenetic reprogramming also presents an opportunity for sensing of environmental cues to dictate the pattern of, for example, remethylation, such that specific functions of the F1 offspring generated from these germ cells are adapted to the perceived environment (Stringer *et al.* 2013). Neat though this idea may be, there are two big potential barriers to its feasibility. First, when sperm are generated, major changes to chromatin architecture (histone replacement by protamines) and DNA methylation occur, such that epigenomic changes originating from fetal life are likely to be erased (Biermann & Steger 2007, Sasaki & Matsui 2008). Second, and more importantly, during fertilization, the zygote undergoes genome-wide epigenetic reprogramming to allow reacquisition of totipotency (Reik 2007, Stringer *et al.* 2013). Therefore, if environmentally influenced epigenetic changes to fetal germ cells in males are to persist so as to change the function of the resulting postnatal individual, they have to be resistant to the reprogramming changes during spermiogenesis and after fertilization (Box 1). There is convincing evidence that this can occur under experimental conditions and growing insight as to the mechanisms involved.

The most informative evidence comes from two studies published in Science (Radford *et al.* 2014, Siklenka *et al.* 2015), which show, respectively, how experimentally induced changes to either DNA or histone methylation during fetal germ cell development leads to changes in sperm that are transmitted inter-generationally and are associated with altered phenotype in the offspring (Box 2). In the first study (Radford *et al.* 2014), pregnant mice were subjected to a 50% restricted calorie diet only during the period of germ cell epigenetic remethylation in the male fetus (e12.5–e18.5). When male fetuses from these underfed mothers grew to adulthood, their sperm exhibited locus-specific DNA hypomethylation, these changes being restricted to nucleosome-retaining regions of the DNA (i.e. regions where histones had not been replaced by protamines). These regions are not random, but contain developmentally important genes (Box 2) – imprinted gene clusters, HOX gene clusters, promoters of developmental transcription and signaling factors (Hammoud *et al.* 2009) and appear to be conserved in mouse and men (Brykczynska *et al.* 2010, Erkek *et al.* 2012). Much of these regions are also resistant to DNA methylation reprogramming in the early embryo and may thus allow persistence of any methylation changes for long enough to affect the phenotypic development of the next (F2) generation. It is intriguing that several of the genes affected by hypomethylation in the study by Radford *et al.* (2014) have known roles in glucose tolerance and metabolism as well as in type 2 diabetes (Box 2), which fits with the notion of fetal adaptive reprogramming to nutritional changes, as well as with other experimental studies described earlier. However, Radford *et al.* (2014) also showed that the differential methylation changes in F1 sperm were not maintained in F2 tissues, but instead,

the latter showed altered expression of genes located close to the genomic regions that had been originally hypomethylated. The authors suggest this may indicate 'sustained alterations in chromatin architecture, transcriptional regulator networks and/or cell type or tissue structure' as a direct consequence of the original epigenetic changes in sperm.

If the example of Radford *et al.* (2014) is illustrative, it suggests that intergenerational epigenetic changes in male germ cells can persist for only one generation, but this may still result in phenotypic changes in the following generation. Whether the latter would then affect the next generation (F3) via male germline transmission, as some have argued does occur (Anway *et al.* 2005), is dependent on there being *de novo* changes to sperm of the F2 animals which are themselves transmissible, a mechanism for which is unclear (Szyf 2015). A detailed study that tried to reproduce the results of Anway *et al.* (2005) demonstrated instead that whilst treatment-induced DNA methylation changes were detectable in pro-spermatogonia of F1 males (that had been directly treatment-exposed *in utero*), no such changes were detectable in pro-spermatogonia of F2 males that had been sired by treatment-exposed F1 males (Iqbal *et al.* 2015). In this regard, this study is very much in keeping with the findings of Radford *et al.* (2014). In a more general sense, other findings that have shown phenotypic changes in F2 offspring that are different from those found in the F1 who were exposed *in utero* to an experimental treatment, might also be considered as fitting with this paradigm (Dean *et al.* 2016).

Another aspect to the studies of Radford *et al.* (2014) is that, during demethylation of primordial germ cells in mice, some areas, termed variably erased CpG islands (CGIs) (VECs; Seisenberger *et al.* 2012) remain methylated at all stages, including in mature oocytes and sperm (Box 2); intriguingly, this can include CGIs in genes involved in insulin-stimulated glucose transport that are associated with type 2 diabetes. More of these methylated CGIs are found in sperm than in oocytes, suggesting that more VECs escape 'methylation reprogramming' in male primordial germ cells (Box 1). These CGIs could also be environmentally sensitive and provide another pathway for intergenerational epigenetic inheritance in mammals (Seisenberger *et al.* 2012). In human PGCs, it has also been shown that specific regions of the genome escape 'genome-wide demethylation', and these include areas that are of known importance in neural development/brain disorders and obesity and, intriguingly, these 'demethylation escapees' showed variable methylation levels between PGCs from individual fetuses (Tang *et al.* 2015*a*,*b*, 2016). These regions provide the means for differential transmission of epigenetic memory intergenerationally. Whilst a detailed analysis and comparison of 'demethylation escapee' regions in mouse and human (Tang *et al.* 2015*a*,*b*) revealed considerable differences (i.e. poor conservation), this also identified genes such as the androgen-responsive cell cycle regulatory gene *TACC2*, which showed resistance to promoter demethylation in human PGCs as was also reported in the homologous gene *Tacc2* in mice from the studies of Radford *et al.* (2014) detailed above, although the actual promoter regions affected differed between mouse and human.

During fetal germ cell de- and re-methylation (Box 1), dramatic changes to chromatin structure involving histone methylation also occur, and these vary considerably between the sexes with much more dramatic increases in histone methylation in male germ cells (Abe *et al.* 2011). The latter authors suggest that these histone changes may provide a framework for gene methylation changes during differentiation of pro-spermatogonia. More generally, it is agreed that histone methylation changes in fetal germ cells is targeted to developmentally important genes, so-called 'bivalent genes,' involved in later cell specification and differentiation (Lesch *et al.* 2013, Voigt *et al.* 2013).

The fundamental importance of correct histone methylation to normal development comes from the second informative study noted earlier (Siklenka *et al.* 2015), which shows that transmissible epigenetic changes in germ cells can be induced without altering DNA methylation, as in Radford *et al.* (2014). The authors used transgenesis to induce a human KDM1A histone lysine 4 demethylase in germ cells in mice and showed that this induced major changes in both coding and non-coding RNA expression in sperm without altering sperm nucleosome structure or DNA methylation. When such animals were mated, they found numerous developmental abnormalities in offspring, and when these offspring were themselves bred, increased incidence of developmental abnormalities were found even out to the F4 generation (Siklenka *et al.* 2015). The intergenerational transmission of the 'effects' occurred in the absence of transmission of the inserted transgene, and thus, was epigenetic, and could be due either to transmission of different RNA profiles in the sperm or more directly to the changes in histone methylation induced originally by the KDM1A transgene. The authors argue that it is highly unlikely that RNA transmission explains the effects and emphasize that their study shows how paternal exposure to an exogenous factor that alters histones, and thus, chromatin architecture, may have consequences for future generations (Siklenka *et al.* 2015). Such changes can presumably be induced during any of the phases in which histone and other chromatin changes occur physiologically, whether during fetal life or during gametogenesis in adulthood.

### *The impact of diet on the epigenome*

The discussion above highlights that during the normal reproductive life-course, there are specific events that *potentially* allow environmental nutritional factors to

influence the epigenome (Fig. 1). Additionally, there is growing evidence from experimental animal studies to show that extremes of diet in parents, whether under- or over-nutrition, can cause changes to the epigenome of germ cells, embryo or fetus, which are associated with altered function and health of the affected individual/offspring, although evidence is mostly lacking for a specific epigenetic cause and effect relationship (Sinclair *et al.* 2010). Even if we accept that human obesity, which is common, can induce similar epigenetic and health consequences as in the experimental animal studies, the key unanswered question is whether it is specific factors (or their lack) in the diet of obese people/animals that causes the epigenetic changes or whether it is the altered metabolic status of the obese individual that is the cause or is it both? Probably many of us will have been told by our mothers when a child that 'you are what you eat', which translated into a scientifically more accurate description is that a balanced, varied diet is widely considered as being the most healthy, as opposed to a fast-food Western diet that is over-rich in calories and deficient in fruit and green vegetables – and of course, it is the latter diet that is associated with obesity. A wide range of compounds derived from plants are capable of altering DNA methylation or causing histone changes (Box 2), albeit mainly in a cancer setting (Shankar *et al.* 2016, Tran *et al.* 2017, Zam & Khadour 2017). The names of some of these compounds will be familiar to us (e.g. circumin, lycopene, quercitin, resveratrol, genistein), but they are mainly classed as phytochemicals and antioxidants and are present in a range of fruit and green vegetables (Shankar *et al.* 2016). It is beyond the scope of this article to consider the epigenetic regulatory properties of these compounds, but it is emphasized that (a) exposure to such compounds would have been common in our evolutionary past and (b) our exposure in the modern world will vary considerably depending on whether we eat a balanced diet or an unbalanced/Western/fast-food diet (Box 2).

One interesting hypothesis, arising from the foregoing discussion, is that because we have evolved whilst eating a plant-rich diet our epigenome is attuned to this because of constant exposure to the phytochemicals in the plants; what a Western diet has done is to disrupt this harmony, possibly with knock-on heritable epigenetic consequences for our children (Krautkramer *et al.* 2016). It may also mean that it is not the contents of a Western diet that does 'epigenetic harm', rather it is what is missing from the diet. Folate, which is a B-vitamin, is an interesting example in this regard, as it is a vital dietary component that we derive naturally from plants and fruits and which can therefore be deficient in a modern Western diet (Steegers-Theunissen *et al.* 2013), especially as it is destroyed by cooking and its circulating levels in pregnant women may be reduced by obesity (Maffoni *et al.* 2017) or by lifestyle factors such as smoking and alcohol consumption (Steegers-Theunissen *et al.* 2013, Drake *et al.* 2015). Folate deficiency, and its relationship to risk of neural tube defects (NTDs) such as spina bifida, has resulted in many countries supplementing common foods (e.g. flour) with a form of folate, folic acid (Gueant *et al.* 2013), which is resistant to cooking. This supplementation has resulted in major reductions in incidence of NTDs, in some cases by 80–90% (Czeizel *et al.* 2011), a change that has also complicated studies into dietary/lifestyle impacts on folate deficiency. Folate/folic acid metabolites play essential roles in so-called 1-carbon metabolism, which underpins the process of DNA methylation (Steegers-Theunissen *et al.* 2013), and these roles and the established relationship between folate deficiency and incidence of NTDs has raised awareness that folate deficiency might have wider 'fetal programming' consequences (Steegers-Theunissen *et al.* 2013, Xu & Sinclair 2015, Joubert *et al.* 2016). This includes possible intergenerational effects as a result of epigenetic changes to germ cells that may result from folate deficiency or from folic acid supplementation (Gueant *et al.* 2013). Indeed, folate is an essential requirement for many of the stages of reproduction in both sexes, and dietary fluctuations in folate could therefore potentially impact the reproductive process at multiple steps (Ebisch *et al.* 2007) (Fig. 1). Several studies have addressed this via experimental dietary manipulations in mice or by studies involving folic acid supplementation in human subjects, such as infertile men.

A double-blind placebo-controlled trial of folic acid supplementation ± zinc sulphate for 6 months in fertile or idiopathic infertile men reported a significant increase in sperm count in the infertile, but not the fertile, group when supplemented with folic acid + zinc sulphate, but no effects with either supplement alone (Wong *et al.* 2002). Whilst there is some further supporting evidence for such effects (Ebisch *et al.* 2007), it is noteworthy that another similar study of men with idiopathic infertility found no effect of 6 month's folic acid supplementation on sperm counts (Aarabi *et al.* 2015), a difference which the latter authors suggested might be due to their infertile group being normospermic, whereas those in the Wong *et al.* (2002) study were oligozoospermic. Experimental studies in mice, comparing a normal diet vs folic acid-deficient and folic acid-supplemented diets suggest that developmental exposure to a folic acid-deficient diet may result in lower sperm counts in adulthood (Swayne *et al.* 2012, Ly *et al.* 2017), an effect that may stem specifically from deficiency during the post-weaning period (Swayne *et al.* 2012). However, supplementation with a very high level of folic acid (20-fold control levels) during development (mating, pregnancy, lactation and post-weaning) resulted in an even bigger decrease in sperm counts in adulthood than did folic acid deficiency, although a 10-fold supplementary dose had no effect (Ly *et al.* 2017). Arguably, the most interesting evidence to emerge from mouse experimental studies is that manipulation of folic

acid levels in the diet throughout development of the male is associated with highly significant changes in DNA methylation in sperm in adulthood, whether in imprinted genes (Ly *et al.* 2017) or genes implicated in the development of chronic diseases such as cancer, diabetes and autistic-spectrum disorders (Lambrot *et al.* 2013). Moreover, there is increased incidence of birth defects in the offspring fathered by males that were exposed to a folic acid-deficient diet during their fetal and postnatal development (Lambrot *et al.* 2013). Now that folic acid supplementation of various common foods is common, it is relatively unlikely that reproductive effects resulting from its deficiency will occur in humans in the developed world. However, it serves as an illustrative example of how gross dietary changes, such as has occurred with a Western style diet, can have far-reaching consequences via the reproductive process, as a result of a change in a specific component of the diet – in this case, the reduced level of folate. An important issue to be resolved in future studies is whether the absence/reduction in other specific factors in a modern Western diet might have comparable intergenerational effects to those evident for folic acid/folate (Box 2).

Although it is interesting to imagine how our diets might impact numerous aspects of our physiology and metabolism via epigenetic mechanisms, the burning question is how important are such effects from a health/disease-risk perspective? It is one thing to show that extremes of parental nutrition, such as famine/starvation in humans or experimental low protein diet in animals, are associated with offspring health changes, but these do not resemble the 'normal' situation in the developed or developing world. However, a small number of studies have begun to address this issue in humans and have generated positive supporting evidence.

A series of studies in rural Gambia (Dominguez-Salas *et al.* 2013, 2014) have been undertaken to evaluate the influence of normal seasonal changes in diet during the rainy season (='hungry' season) and the dry season (='harvest' season) on the blood levels of 13 biomarkers of key components of the 1-carbon metabolism cycle (e.g. choline, betaine, folate, methionine, vitamins B-6 and B-12) in women around the time that they became pregnant. These showed consistent changes in bioavailability of 8 out of 13 factors according to the season of conception in the studied women, leading the authors to conclude that 'naturally occurring seasonal variations in food consumption patterns have a profound effect on methyl-donor biomarkers status' (Dominguez-Salas *et al.* 2013). What makes the studies ingenious is that they then studied the DNA methylation status at six established metastable epialleles (MEs) in peripheral blood lymphocytes and hair follicles from the offspring resulting from these pregnancies (Dominguez-Salas *et al.* 2014). This showed, first, that ME methylation status was highly correlated ($r=0.72$; $n=167$) in peripheral blood lymphocytes and hair follicles from the offspring and varied in a consistent manner for all MEs according to the season of conception. Moreover, levels of several of the measured biomarkers at the time of conception predicted the mean ME methylation with the direction of effect being consistent with the biological relationship between the biomarkers in question and DNA methylation. It is emphasized that the effect sizes in mean % DNA methylation for a given ME in offspring conceived in the different seasons were not large, but the significance is that such changes occurred under conditions that are normal, as opposed to extreme (e.g. famine). Importantly, such conditions are likely to have been a common feature of our evolutionary past, so in many respects, these Gambian studies provide strong support for the underlying central hypothesis of the present review.

One of the MEs identified in the Gambian studies as being variably methylated according to season of conception was the tumour suppressor gene *VTRNA2-1*, which plays a role in innate immunity amongst other functions (Silver *et al.* 2015). This gene was identified independently as a metastable epiallele in a screening study for variably methylated genes, and its methylation status shown to vary between normal Caucasian and Asian adults similar to that shown in the Gambian individuals (Silver *et al.* 2015). Whether such variation in specific gene methylation results in increased risk of disease was not established in the aforementioned studies, but evidence from another study of methylation in a variably methylated region (VMR) of the pro-opiomelanocortin (*POMC*) gene provides support for this possibility (Kuhnen *et al.* 2016). *POMC*-VMR methylation status in brain neurons involved in appetite control was found to be strongly associated with individual BMI in different ethnic cohorts, including in individuals from the Gambian studies described earlier. Using the latter, authors showed that *POMC*-VMR methylation status was partly inherited from fathers, but not from mothers, but its methylation was strongly affected by season of conception similar to the other epialleles described earlier (Dominguez-Salas *et al.* 2013, 2014, Kuhnen *et al.* 2016). Thus, maternal diet at conception can modify inherited DNA methylation status, at least for the *POMC* gene, illustrating the adaptability of the overall process in humans in real-world contexts.

Plant-derived vitamins and phytochemicals are only one aspect of diet, and do not provide essential protein, fats and carbohydrates, raising the question of whether these can also affect the epigenome. This is more complex and less well studied, but there is growing evidence from a variety of sources that histone modifications such as acetylation and methylation can be influenced by dietary carbohydrates (Krautkramer *et al.* 2016) possibly via interactive effects with the gut microbiome (Liu *et al.* 2016). The underlying pathways of effect are not so well studied, but an obvious possibility is that dietary factors target the activity of the enzymes that regulate histone

modifications. For example EZH2, which is responsible for the repressive histone methylation H3K27me3, can be affected by types of fats in the diet (Rodriguez-Miguel *et al.* 2015), by omega-3 polyunsaturated fatty acids (Dimri *et al.* 2010), low dietary protein intake (Fontana *et al.* 2013), by isoflavones (Kanwal *et al.* 2016) or by sulforafane (Fisher *et al.* 2016), which is a phytochemical derived from broccoli and brussels sprouts with known tumour-suppressing properties. It is a huge jump from experimental studies such as those above to considering whether variation in maternal diet in human pregnancy can influence the pattern and extent of DNA or histone methylation changes in fetal germ cells in such a way that it can alter later development and/or pass on this epigenetic change to the next generation. As the foregoing discussion makes clear, such effects are certainly feasible from the perspectives of exposure (diet) and opportunity (germ cell epigenetic remodeling periods), but undertaking meaningful studies in humans is plagued with complexities, whether of the diet itself or of the numerous confounding factors (genetic, lifestyle, own development) that are likely to obstruct interpretation.

## Future prospects and concluding remarks

If one accepts the premise that reproduction is our reason for being, a reasonable following conclusion is that we have been designed (or have been shaped during evolution) with this one purpose in mind; thus, the reproductive process is the pivot for the rest of our physiology. For those involved in non-reproductive biomedical research, this simple chain of logic will likely be alien and perhaps offensive, but in my opinion the evidence to support this notion is overwhelming. Only when one begins to view the world via this reproductive prism, can you gain a proper perspective, and then to ask the correct questions or to interpret data with the reproductive process at the forefront of your mind. My hope is that this brief overview will encourage others to think in the way that I have outlined and thus help to elevate 'reproductive biology' back onto the pedestal from which it has been dislodged. This I consider to be the easy task. The more difficult task is to persuade readers that the growing evidence for fetal programming and for inter-generational effects of diet may be 'reproductively purposeful', that it is evidence for rapid adaptive (epigenetic) mechanisms to better fit the future offspring to its nutritional environment so as to give it a reproductive advantage. I hope that the reader will agree with me that, at least at the descriptive level, there is abundant supporting evidence for this hypothesis, including in humans. However, whether the evidence that I have presented is actually indicative of cause and effect with a reproductive purpose requires numerous assumptions; and of course, assumptions are ultimately the ruin of most hypotheses.

The key issue in my opinion is whether the epigenetic changes induced in offspring by paternal diet are intentionally adaptive (for the offspring) or are simply the incidental downstream consequence of a change of paternal diet or metabolism. The latter presumes that nature is not so clever (almost a 'sitting duck'), whereas the former interpretation (my hypothesis) presumes that nature is ingenious. But how do we decide which is correct? For the moment, I consider that this dilemma cannot be resolved evidentially, because we lack any indisputable complete chain of evidence that connects a specific change in paternal diet to a specific epigenetic change(s), which can be shown to be transmitted to the next generation and to specifically cause biological changes in the offspring that are specific to the original dietary change in the father. Obtaining such information is enormously challenging, not least because the available evidence suggests that, at least for paternally mediated effects, dietary or experimentally induced changes in the father's sperm epigenome (e.g. miRNA profile, DNA methylome) are not recapitulated in the offspring or grand-offspring despite the fact that phenotypic changes are evident in these offspring as a result of the paternal dietary insult (Fullston *et al.* 2013, 2016, Siklenka *et al.* 2015, Chambers *et al.* 2016). Dissecting out cause and effect in such inter-generational studies, and designing the appropriate controls at each step, is a Herculean task, that is further limited by our present poor understanding of epigenetic processes, their regulation and integration. We can be certain that this understanding will grow exponentially and hopefully this will facilitate a more targeted research approach to 'the chain of effect' issue just outlined. More studies along the lines of investigating dietary effects in real-world situations, such as those conducted in rural Gambian women as discussed earlier (Dominguez-Salas *et al.* 2013, 2014, Silver *et al.* 2015), is the most obvious way to make progress in this area.

An ever-present issue, and a big one, is the extent to which the mouse experimental inter-generational studies can be translated to us humans – thus, even if the hypothesis is correct, how big an influence is, for example, paternal diet on a father's offspring (Box 2)? If the effects are small, why should we bother? This leads to my biggest reservation, which is whether anyone will be bothered to even evaluate this hypothesis – in other words to care whether or not reproduction has an intergenerational adaptive influence in our lives that extends far beyond the simple established transfer of DNA from parents to child. In this regard, the Gambian studies discussed earlier could also lead the way, if researchers can be motivated to investigate if seasonal dietary changes in mothers that are associated with DNA methylation changes in offspring, are also associated with reproductive changes in offspring. Other than this, I foresee that the focus will be on how diseases in our children are caused – and if aspects of paternal (or maternal) diet are proven to cause disease

in our children, this will be the trigger to understand the epigenetic mechanisms involved. Whether or not this has an underlying reproductive purpose is a question that is rather unlikely to be asked, and in science, we all know that one only gets answers when one asks the right question. Hence, why I am posing this question now via this review. In doing so, my hope is that it will stimulate young researchers to see the truly big picture and to seek such an answer. If reproduction really is our biological reason for being, and if nature is as ingenious as the evidence all around us indicates it is, then framing our questions with this in mind will increase the chances of researchers asking the right questions. Whether this will prove the hypothesis right or wrong does not matter, what matters is to get an answer.

## Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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